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glucose metabolism through the PPP to purine synthesis. Mitsuishi et al. (2012) therefore argue that a proliferative signal provided by the PI3K-Akt pathway is required to increase flux through the PPP in an Nrf2-dependent fashion and provide evidence that Akt is activated in mouse forestomach. The idea that increased PI3K-Akt signaling in the liver allows Nrf2 to induce *G6pd*, *Tkt*, *Taldo1*, *Pgd*, and *Me1* was confirmed by making liver-specific Pten and Keap1 double-knockout mice.

In summary, Mitsuishi et al. show that under the direction of PI3K-Akt signaling, Nrf2 regulates the expression of genes involved in the PPP, generation of NADPH, and synthesis of purine nucleotides. They have expanded our knowledge of Nrf2 functions from increasing cytoprotection to the regulation of metabolism and the synthesis of macromolecules, thereby providing an explanation of how Nrf2 supports cell proliferation. A number of critical issues remain unresolved. The mechanism by which Nrf2-mediated induction of metabolic genes requires both inhibition of Keap1 and activation of PI3K-Akt, as opposed to induction of antioxidant and detoxication genes, which requires only inhibition of Keap1, is unclear. It is also not known how Nrf2 cooperates with other transcription factors implicated in metabolic reprogramming in tumor cells, including p53, Myc, and HIF1α (Kroemer and Pouyssegur, 2008; Ward and Thompson, 2012), to maintain high rates of glycolysis and increase cell proliferation. It remains to be established whether activation of Nrf2 along with the PI3K-Akt pathway is sufficient to promote carcinogenesis. Lastly, while inhibition of Nrf2 as a cancer chemotherapeutic strategy is an attractive proposition, it may be unwise to reduce PPP metabolic flux, given that the pathway provides an important defense for neurons and astrocytes against oxidative damage and neuronal death (Fernandez-Fernandez et al., 2012). Accordingly, it is becoming clear that Nrf2, in combination with growth factor signaling, provides a critical interface between oxidative stress sensing and metabolic reprogramming of cells.

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The Mitochondrial Pyruvate Carrier: Has It Been Unearthed at Last?

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The mitochondrial pyruvate carrier (MPC) is essential for several major pathways of carbohydrate, fat, and amino acid metabolism, yet its molecular identity has remained elusive. Two recent papers in *Science* (Herzig et al., 2012; Bricker et al., 2012) implicate three newly identified inner mitochondrial membrane proteins as MPC components.

Pyruvate lies at the heart of carbohydrate, fat, and amino acid metabolism and is usually produced in the cytoplasm before being transported into the mitochondria for further metabolism (Figure 1). Transport is mediated by the mitochondrial pyruvate carrier (MPC) whose existence was confirmed in 1974 by the discovery of a potent and specific inhibitor, α-cyano-4-hydroxycinnamate (CHC). Subsequently, its substrate and inhibitor specificity and roles in metabolism were extensively investigated (Halestrap et al., 1980). Transport of most metabolites across the inner mitochondrial membrane (IMM) involves members of the mitochondrial carrier family (MCF; 53 members in humans), which are usually 30–35 kDa in size and have six transmembrane domains (TMDs) (Palmieri and Pierri, 2010). In 1981 the CHC analog UK5099 (K_i 5 nM)

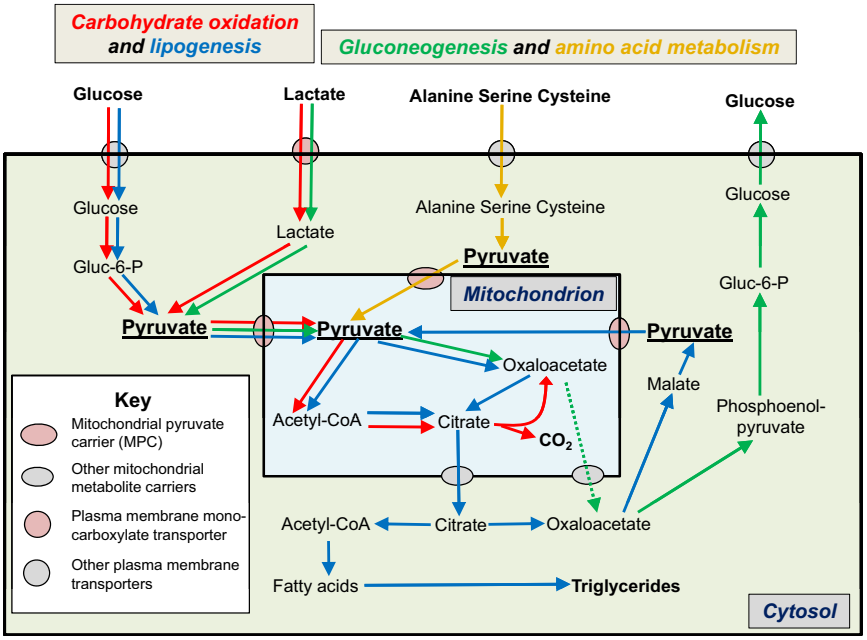

Figure 1. Major Pathways of Mammalian Carbohydrate, Fat, and Amino Acid Metabolism that Require the MPC

Pathways are color coded as indicated. The dotted green line represents the indirect pathway of oxaloacetate transport out of the mitochondria. Note that the MPC is also used for the transport of the ketone body, acetoacetate, and is known to be inhibited by phenylpyruvate and α-ketoisocaproate. These monocarboxylates accumulate in phenylketonuria and maple syrup urine disease, respectively, and may account for the perturbation of brain energy metabolism and development associated with these diseases (Halestrap et al., 1980).

was shown to protect a 15 kDa inner mitochondrial membrane (IMM) protein from labeling with [^{3}H]-N-phenylmaleimide (Thomas and Halestrap, 1981) but the molecular identity of the labeled protein was not established. Another attempt to identify the MPC involved measuring pyruvate transport into mitochondria from strains of yeast (*Saccharomyces cerevisiae*) in which each member of the MCF without an ascribed function had been deleted. Only in the case of *YIL006w* was inhibitor-sensitive pyruvate transport totally abolished, implying that this gene encoded the MPC (Hildyard and Halestrap, 2003), although Palmieri's group subsequently reported that *YIL006w* encoded the mitochondrial NAD^+ transporter (Todisco et al., 2006). Thus, after nearly 40 years, the identity of the MPC remains a mystery—but two recent papers in *Science* (Herzig et al., 2012; Bricker et al., 2012) may have changed this.

Herzig et al. (2012) deleted yeast genes encoding the three members of a family of IMM proteins of unknown function that are predicted to contain three TMDs and are conserved from yeast to man. Deletion of these genes impaired mitochondrial lipoic acid production from pyruvate but not from valine or leucine. Lipoic acid is synthesized from mitochondrial acetyl-CoA, itself produced from pyruvate by oxidative decarboxylation catalyzed by pyruvate dehydrogenase (PDH). Indeed deletion of PDH produced a similar phenotype, yet PDH activity was normal in leucine- or valine-treated cells where lipoic acid is synthesized independently of PDH. This led the authors to conclude that this protein family was required for pyruvate transport into mitochondria. They named the gene products Mpc1, Mpc2, and Mpc3, and substantiated their role by measurement of UK5099-inhibitable [^{14}C]-pyruvate uptake into isolated mitochondria from the different yeast strains. The mouse genome contains two orthologs, mMpc1 and mMpc2, and their expression rescues the yeast deletion mutants. Final confirmation that mMpc1 and mMpc2 facilitate pyruvate transport was provided by expressing them in *Lactococcus lactis* and demonstrating UK5099-sensitive pyruvate uptake only when both were expressed together. The authors conclude that mMpc1 and mMpc2 are essential components of the MPC in mammalian mitochondria.

Bricker et al. (2012) reached a similar conclusion. The growth media requirements and metabolomic analysis of yeast Mpc1 and Mpc2 deletions implicated these proteins in mitochondrial pyruvate transport, and the authors substantiated this role by assaying UK5099-inhibitable [^{14}C]-pyruvate uptake into mitochondria. The metabolic requirements of *Drosophila melanogaster* lacking orthologs of Mpc1 and Mpc2 were consistent with these proteins playing the same role in fly mitochondria. In addition, knockdown of the mammalian orthologs in cultured mouse embryonic fibroblasts inhibited pyruvate-dependent respiration. Furthermore, in three unrelated families, children with lactic acidosis and hyperpyruvatemia showed mutations in a conserved region of human Mpc1, while their fibroblasts exhibited impaired pyruvate-dependent respiration that was restored by expression of wild-type Mpc1.

Together, these two papers demonstrate that Mpc1 and Mpc2 play a critical role in mammalian mitochondrial pyruvate metabolism, but do they mediate transport? Mitochondrial pyruvate transport is very fast and occurs on top of a significant rate of inhibitor-insensitive free diffusion. In addition, pyruvate is metabolized rapidly within the mitochondria. For this reason, accurate measurement of pyruvate transport requires the use of low temperatures, inhibition of metabolism, and rapid termination of transport by centrifugation (Halestrap et al., 1980). Given the technical challenges in measuring pyruvate transport, it remains possible that the [^{14}C]-pyruvate uptake measured in these two papers actually represents pyruvate metabolism rather than transport. Indeed, Herzig et al. (2012) showed that PDH activity was reduced in mitochondria from the Mpc mutants due to the decreased mitochondrial lipoamide, and this would be expected to reduce accumulation of pyruvate metabolites. Furthermore, the MPC inhibitor UK5099, at the very high concentration used by the authors, may also inhibit mitochondrial respiration. The most convincing data is undoubtedly that demonstrating increased pyruvate

uptake into *Lactococcus lactis* expressing mMpc1 and mMpc2, although the rates measured were very slow.

Despite these caveats, it is likely that Mpc1 and Mpc2 do represent components of the MPC. Indeed, this might explain the earlier identification of a 15 kDa protein as the binding site for UK5099 in rat heart and liver IMMs (Thomas and Halestrap, 1981). Interestingly, this inhibitor is known to attack a critical thiol group on the MPC and there is a conserved cysteine in TMD3 of Mpc1 but not Mpc2. The authors suggest that with only three TMDs, the Mpc members are most likely to form an active transporter by associating in a multisubunit complex, and both papers present coimmunoprecipitation data in support of this notion. Equally possible is that there is a member of the MCF family that interacts with Mpc1 and Mpc2 to mediate mitochondrial pyruvate transport. This would be consistent with blue native-polyacrylamide gel electrophoresis data that suggest the Mpc1 and Mpc2 exist as part of a 150 kDa complex (Bricker et al., 2012). Interestingly, the plasma membrane transport of pyruvate and lactate is mediated by a 12-transmembrane protein that requires a single transmembrane ancillary protein (Halestrap, 2012). Future work will be needed to clarify which, if any, of these explanations is correct, and ultimate proof will require measurement of pyruvate transport by the purified protein(s) reconstituted into proteoliposomes. It will also be important to establish whether the MPC is regulated in response to known changes in pyruvate metabolism such as in the starved-to-fed transition and during exercise and whether this regulation is transcriptional or by covalent modification. Another interesting area to explore is whether MPC expression is reduced in tumor cells whose energy metabolism is largely glycolytic.

In conclusion, the data of Herzig et al. (2012) and Bricker et al. (2012) may not provide a complete picture of the identity of the MPC, but they do represent a major step forward and provide a much-needed stimulus for future work on its roles and regulation.

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